REMARKS

Applicants have amended Claims 1, 2, 8, 9, 21, 30, 31, 37 and 38. Enabling support for the amendments can be found in the application as filed, and therefore no new matter is contained in the amendments. Reconsideration of the present application and allowance of resulting Claims 1-9, 21-25, 27, and 30-38 is respectfully requested in view of the amendments and following remarks.

I. Claim Objections

The Office Action rejects Claims 1, 8-9, 30 and 37-38 for encompassing non-elected subject matter, namely "regulatable" promoters other than somatic tissue-preferred promoters. Applicants have amended Claims 1, 8-9, 30 and 37-38 to recite a "somatic tissue-preferred promoter," and have amended Claims 9 and 38 to recite various types of somatic-tissue preferred promoters, and submit that these amendments overcome the objection.

The Office Action objects to Claims 21-25 for depending upon non-elected claims. Applicants have amended Claim 21 to depend solely upon elected claims, with the understanding that if the elected claims are allowed, that the non-elected claims can still be examined. Claims 22-25 are dependent upon Claim 21, and are therefore similarly limited to the elected species. Applicants submit that the amendment overcomes the objections.

The Office Action objects to Claims 21-25 under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim can only depend upon another multiply dependent claim in the alternative. Applicants have amended Claim 21 to depend in the alternative. Claims 22-25 depend upon Claim 21, and therefore now similarly depend in the alternative. Applicants submit that this amendment overcomes the objection.

For at least the foregoing reasons, Applicants respectfully request reconsideration and removal of the rejections and allowance of Claims 1, 8-9, 30 and 37-38.

II. Claim Rejections under 35 U.S.C. § 112, first paragraph, written description requirement

The Office Action has rejected Claims 1-9, 21-25, 27, and 30-38 under 35 U.S.C. §112, first paragraph as failing to comply with the written description requirement. The Office Action states that "the claims are broadly drawn to polynucleotides comprising a multitude of somatic tissue-preferred promoters, including leaf-, root-, meristem-, and tuber-preferred promoters, form a multitude of plant and gene sources and of a multitude of sequences; methods for their use; and plant cells and plants transformed therewith. In contrast, the specification provides no guidance for the characterization or description of any somatic tissue-preferred promoter in terms of sequence or gene source, and no plant cell or plant transformed with any of said promoters was reduced to practice." Applicants respectfully traverse this rejection as follows.

Claims 1 and 30 of the present application have been amended to incorporate the limitations that the recombinase be an irreversible recombinase, and that the recombination sites be corresponding recombination sites. The terms "irreversible recombinase", "recombination sites" and "corresponding recombination sites" are explained in detail in the instant specification at, for example, page 10, paragraph 31, page 10, paragraph 27, and page 11 paragraph 32. These amendments narrow Claims 1 and 30 and also narrow the remaining dependent claims, 2-9, 21-25, 27, and 31-38.

Applicant is not required to isolate and identify every tissue-preferred promoter. Applicant has developed self-excising polynucleotides useful in eukaryotic tissues. The exact structure of the somatic-tissue preferred promoter will differ with the promoter selected,

however, the use of a particular promoter is not critical to the nature of the invention. Any somatic tissue- preferred promoter can be used in the instant invention. The identification of promoters for use in the self-excising polynucleotide is routine for one of ordinary skill in the art. As examples of tissue-preferred promoters, the specification sets out at paragraphs 51 and 52:

> Other suitable tissue-preferred or organ-preferred promoters include the napin-gene promoter from rapeseed (U.S. Patent No. 5,608,152), the USP-promoter from Vicia faba (Baeumlein et al. 1991 Mol Gen Genet. 225(3):459-67), the oleosin-promoter from *Arabidopsis* (PCT Application No. WO 98/45461), the phaseolin-promoter from *Phaseolus vulgaris* (U.S. Patent No. 5,504,200), the Bce4-promoter from Brassica (PCT Application No. WO 91/13980) or the legumin B4 promoter (LeB4; Baeumlein et al. 1992 Plant Journal, 2(2):233-9) as well as promoters conferring seed specific expression in monocot plants like maize, barley, wheat, rye, rice, etc. Suitable promoters to note are the lpt2 or lpt1-gene promoter from barley (PCT Application No. WO 95/15389 and PCT Application No. WO 95/23230) or those described in PCT Application No. WO 99/16890 (promoters from the barley hordein-gene, rice glutelin gene, rice oryzin gene, rice prolamin gene, wheat gliadin gene, wheat glutelin gene, oat glutelin gene, Sorghum kasirin-gene and rye secalin gene).
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> Additionally, the tissue-specific E8 promoter from tomato is particularly useful for directing gene expression so that a desired gene product is located in fruits. *See, e.g.*, Lincoln *et al.*, 1988 Proc. Nat'l. Acad. Sci. USA 84: 2793-2797; Deikman *et al.*, 1988 EMBO J. 7: 3315-3320; Deikman *et al.*, 1992 Plant Physiol. 100: 2013-2017. Other suitable promoters include those from genes encoding embryonic storage proteins. Additional organ-specific, tissue-specific and/or inducible foreign promoters are also known (*see, e.g.*, references cited in Kuhlemeier *et al.*, 1987 Ann. Rev. Plant Physiol. 38:221), including those 1,5-ribulose bisphosphate carboxylase small subunit genes of *Arabidopsis thaliana* (the "ssu" promoter), which are light-inducible and active only in photosynthetic tissue, anther-specific promoters (EP 344029), and seed-specific promoters of, for example, *Arabidopsis thaliana* (Krebbers *et al.*, 1988 Plant Physiol. 87:859). Exemplary green tissue-specific promoters include the maize phosphoenol pyruvate carboxylase (PEPC) promoter, small submit ribulose bis-carboxylase promoters (ssRUBISCO) and the chlorophyll a/b binding protein promoters. The promoter may also be a pith-specific promoter, such as the promoter isolated from a plant TrpA gene as described in International Publication No. W0/93/07278.

If the specification is read in light of the knowledge and level of skill in the art, the specification discloses the steps of the claimed process and the elements of the claimed composition. As stated by the Federal Circuit "an inventor is not required to describe every detail of his invention. An applicant's disclosure obligation varies according to the art to which the invention pertains." *In re Hayes Microcomputer Products Inc. Patent Litigation*, 982 F2d. 1527, 1534-35 (Fed. Cir. 1992). Certain areas of biotechnology such as the art of recombinant DNA technology are generally regarded as highly predictable with a high level of skill, such that the selection of a promoter that is somatic tissue-preferred is well within the routine skills of one skilled in the art. The prior art provides numerous examples of somatic tissue-preferred promoters from a wide variety of eukaryotes, including animals. In the instant application, somatic tissue-preferred promoters and irreversible recombinases are described in the specification, are conventional in the art, and are known to one of ordinary skill in the art. The selection of somatic tissue-preferred promoters is sufficiently developed so as to put one of skill in the art in possession of the steps of the method and the elements of the composition. In other words, one skilled in the relevant art would understand what is intended by the claimed invention and how to carry it out.

For at least the foregoing reasons, Applicants respectfully request reconsideration and removal of the rejection and allowance of Claims 1-9, 21-25, 27, and 30-38.

III. Claim Rejections under 35 U.S.C. § 112, first paragraph, enablement requirement

The Office Action has rejected Claims 1-9, 21-25, 27, and 30-38 under 35 U.S.C. §112, first paragraph as failing to comply with the enablement requirement. The Office Action states that "the specification, while being enabling for claims limited to polynucleotides comprising a plant somatic tissue-specific promoter ligated to a gene encoding a theta [sic] C31

recombinase for the controlled excision of desired trait polynucleotides in plants, wherein the polynucleotides are flanked by recombination sites which are recognized by the theta [sic] C31 recombinase, does not reasonably provide enablement for claims broadly drawn to any somatic tissue-specific promoter functional in non-plant organisms such as animals, or any other recombinase-encoding gene, or the use of the theta [sic] recombinase with non-corresponding recombination recognition sites such as loxP or FRT. . . . Claims 1-2, 6-9, 21-25, 27, 30-31, and 35-38 are broadly drawn to polynucleotides encoding a multitude of recombinases from a multitude of sources, including reversible and irreversible recombinases, and their use to effect controlled excision of transgenes. Claims 1-6, 8, 21-25, 27, 30-35 and 37 are broadly drawn to polynucleotides comprising any developmentally regulatable promoter functional in any organism including animals. All the claims are broadly drawn to any of a multitude of recombination sites. No guidance is provided for polynucleotides comprising any animal-functional promoters or methods of animal transformation, and no guidance is provided for polynucleotides comprising any other type of recombinase gene other than the theta [sic] recombinase gene for controlled and permanent transgene excision in transformed plants. In addition, no guidance is provided for successful recombinase-mediated excision when non-corresponding recombination recognition sites are used." Applicants respectfully traverse this rejection as follows.

In determining whether a patent application is enabled, it must be considered whether a person of ordinary skill in the art could practice the invention without "undue experimentation." *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed Cir. 1998). This judicially-created test sets forth eight Wands factors that are to be considered in determining whether a patent application is enabled:

1. the breadth of the claims,

2. the nature of the invention,
3. the state of the prior art,
4. the level of skill possessed by one of ordinary skill in the art,
5. the level of predictability in the art,
6. the amount of direction provided in the application,
7. the existence of working examples in the specification, and
8. the quantity of experimentation needed to make or use the invention based on the content of the disclosure.

While not all of these factors must be considered in making a determination of enablement, the factors must be considered as a group. *Enzo Biochem., Inc. v. Calgene, Inc.*, 188 F.3d 1362, 52 USPQ2d 1129 (Fed. Cir. 1999). Because the Wands factors must be considered as a whole, an application that is deficient with respect to one or more factors may still be enabled. For example, providing extensive guidance in the specification and disclosing multiple working examples may compensate for a relatively high level of unpredictability or a low level of skill in the art.

Applicant concedes that some experimentation will be involved in determining whether a particular construct is effective or optimal in a particular plant or animal. However, this type of optimization is routine for one of ordinary skill in the art, and does not constitute undue experimentation. Applicant notes that the quantity of experimentation needed to be performed is only one factor involved in determining whether undue experimentation is required to make and use the invention. "Time and difficulty of experiments are not determinative if they are merely routine." MPEP §2164.06. Applicants submit that one of skill in the art would be able to make and use the claimed invention in a variety of cells types using the application as a guide. For example, the specification teaches that methods of introducing the constructs into a variety of cells may be accomplished using any technique known to those of skill in the art, including biolistic methods, electroporation, microinjection, PEG-mediated transformation,

Agrobacterium mediated transformation, liposome-based DNA delivery, and viral vectors. While *Agrobacterium* mediated transformation is limited to use in plant cells, the other techniques are commonly used with animal cells. Transformation and integration of DNA can be selected for using selectable markers as indicated in the instant specification. The integration patterns can be analyzed using methods well known in the art, including the analysis of DNA from the transformed cells, or the analysis of cells from an organism derived from the transformed cell, such as from a regenerated plant or its progeny, or from a transgenic or chimeric animal. These steps are all routine to one of ordinary skill in the art and do not constitute undue experimentation.

Claims 1 and 30 have been amended to recite irreversible recombinases and irreversible recombination sites. In addition, the recombination sites are "corresponding recombination sites", such that the recombination sites will serve as a substrate for recombination for the specific recombinase used, and will recombine with each other to form a hybrid recombination site.

The claims are directed towards the use of irreversible recombinases. Irreversible recombinases are not specific to plants or animals, and can be used across species. For example, phiC31 has been effective in performing site-specific recombination in tobacco (see Examples 4 and 5 in PCT Publication No. WO 01/07572A2: *DNA Recombination in Eukaryotic Cells by the Bacteriophage PhiC31 Recombination System*, enclosed herewith), and has also been effective in mammalian cells to integrate a nucleic acid (Groth *et al.*, 2000, Proc. Natl. Acad. Sci. U.S.A., "*A phage integrase directs efficient site-specific integration in human cells*" 97(11):5995-6000, enclosed herewith). In addition, other irreversible recombinases have been shown to be effective in mammalian cells in integrating nucleic acids (see, for example, Young *et al.*, 2000, J. Virol., "*Roles of adeno-associated virus Rep protein and human*

chromosome 19 in site-specific recombination" 74(9):3953-66; Lamartina *et al.*, 2000, J. Virol, "*Characteristics of the adeno-associated virus preintegration site in human chromosome 19: open chromatin conformation and transcription-competent environment*" 74(16):7671-7; and Dutheil *et al.*, 2000, Proc Natl Acad Sci U.S.A., "*Adeno-associated virus site-specifically integrates into a muscle-specific DNA region*" 97(9):4862-6, enclosed herewith.).

The Office Action states that "no guidance is provided for polynucleotides comprising any animal-functional promoters or methods of animal transformation. . ." Applicant submits that the identification and selection of promoters that are functional in the somatic tissues of animals is routine and well within the abilities of one of skill in the art. The prior art has identified multiple animal somatic tissue-preferred promoters. For example, the 5' flanking region of the human endothelial nitric oxide synthase (eNOS) promoter, approximately 1600 bp in length, drives expression in a subset of endothelial cells, cardiomyocytes and vascular smooth muscle cells (see Guillot *et al.*, 2000, Physiol. Genomics, "*Targeting of human eNOS promoter to the Hprt locus of mice leads to tissue-restricted transgene expression*" 2(2):77-83, enclosed herewith); the 2.1 kb upstream sequences of the tyrosine kinase receptor *Tie2* gene promote endothelial cell-specific expression in embryonic development, and sequences located in intron 1 of the Tie2 gene are required for endothelial-specific expression throughout adult life (see Evans *et al.*, 2000, Physiol. Genomics., "*Targeting the Hprt locus in mice reveals differential regulation of Tie2 gene expression in the endothelium*" 2(2): 67-75, enclosed herewith); and a 2.5-kb region of the perinatal MHC gene promoter/enhancer is sufficient to direct expression in regenerating fast and slow skeletal muscle (see Swoap *et al.*, 2000, Am. J. Physiol. Cell Physiol., "*The calcineurin-NFAT pathway and muscle fiber-type gene expression*" 279: C915-C924, enclosed herewith).

In addition, as noted above, the specification teaches methods of introducing constructs into a variety of cells using any technique known to those of skill in the art, including biolistic methods, electroporation, microinjection, PEG-mediated transformation, liposome-based DNA delivery, and viral vectors such as papillomaviral, retroviral and adeno-associated viral vectors. All of these techniques have been used successfully to transform animal cells by those of ordinary skill in the art.

The Office Action further states "it is well-known in the art that tissue-specific and developmentally regulated promoters will not function in divergent tissues, since the chemical stimuli required for their activation is not present. Thus, animal-derived tissue-specific or developmentally regulated promoters will not function in transformed plant tissue." Applicant concedes that different tissue specific promoters will generally be used for plants than will be used in animals. However, as described above, the selection and optimization of tissue-specific promoters is routine for one of ordinary skill in the art, and does not constitute undue experimentation.

For at least the foregoing reasons, Applicants respectfully request reconsideration and removal of the rejections and allowance of Claims 1-9, 21-25, 27, and 30-38.

IV. Claim Rejections under 35 U.S.C. §102

The Office Action rejected Claims 1-2, 6-9, 21-23, 30-31 and 35-38 under 35 U.S.C. §102(b) as being anticipated by WO 97/37012 to Commonwealth. The Office Action states that Commonwealth teaches "polynucleotides comprising a selectable marker or a morphology-altering/regeneration enhancing "desired trait polynucleotide" and a Cre recombinase polynucleotide, operably linked to the leaf-specific rbcS (RUBISCO) promoter, all

flanked by directed oriented loxP recombination sites; for the excision of unwanted transgenes which either pose an environmental or health threat, or which cause an unwanted metabolic or genetic load, after their initial expression in particular cell types; and transformed tobacco cells and plants."

Claims 1 and 30 have been amended to more particularly point out the invention and require that the recombinase is an irreversible recombinase and recombination sites are irreversible corresponding recombination sites. Commonwealth does not teach the use of an irreversible recombinase, and does not teach the use of irreversible recombination sites. Claims 2, 6-9, 21-23, 31 and 35-38 are dependent on Claims 1 and 30, and as such, are now distinguished from the cited §102 reference. For at least these reasons, Applicants submit that the amendments overcome the rejections and respectfully request reconsideration and allowance of the amended claims.

V. Claim Rejections under 35 U.S.C. §103(a)

The Office Action rejected Claims 1-2, 6-9, 21-22, 30-31 and 35-38 under 35 U.S.C. §103(a) as being unpatentable over WO 97/37012 to Commonwealth in view of Arntzen *et al.* (U.S. 5,972,935). The Office Action also rejected Claims 1-2, 6-9, 21-22, 25, 27, 30-31 and 35-38 under 35 U.S.C. §103(a) as being unpatentable over WO 97/37012 to Commonwealth in view of Sederoff *et al.* (U.S. 4,886,937).

Claims 1 and 30 have been amended to more particularly point out the invention and require that the recombinase is an irreversible recombinase and recombination sites are corresponding recombination sites. Support for the amendments in Claims 1 and 30 can be found in the specification as filed. None of the cited §103 prior art references teaches or suggests the use of an irreversible recombinase or the use of corresponding recombination sites.

Claims 2, 6-9, 21-22, 25, 27, 30-31 and 35-38 are dependent on Claims 1 and 30, and as such, are now distinguished from the cited §103 references.

Accordingly, Applicants submit that the amendments overcome the §103(a) rejections and respectfully request reconsideration and allowance of the amended claims.

For at least the foregoing reasons, Applicants respectfully request reconsideration and removal of the rejections and allowance of Claims 1-9, 21-25, 27, and 30-38. The foregoing is submitted as a full and complete Response to the Office Action mailed June 6, 2003. No additional fees are believed due; however, the Commissioner is hereby authorized to charge any additional fees that may be required, or credit any overpayment to Deposit Account No. 19-5029. This Response places all claims in the present application in condition for allowance, and such action is courteously solicited. The Examiner is invited and encouraged to contact the undersigned attorney of record if such contact will facilitate an efficient examination and allowance of the application.

Respectfully submitted,

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